

FLOWERING NEWSLETTER REVIEW

***FLC* or not *FLC*: the other side of vernalization**

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Abstract

Vernalization is the promotion of the competence for flowering by long periods of low temperatures such as those typically experienced during winters. In *Arabidopsis*, the vernalization response is, to a large extent, mediated by the repression of the floral repressor *FLC*, and the stable epigenetic silencing of *FLC* after cold treatments is essential for vernalization. In addition to *FLC*, other vernalization targets exist in *Arabidopsis*. In grasses, vernalization seems to be entirely independent of *FLC*. Here, the current understanding of *FLC*-independent branches of the vernalization pathway in *Arabidopsis* and vernalization without *FLC* in grasses is discussed. This review focuses on the role of *AGL19*, *AGL24*, and the *MAF* genes in *Arabidopsis*. Interestingly, vernalization acts through related molecular machineries on distinct targets. In particular, protein complexes similar to *Drosophila* Polycomb Repressive Complex 2 play a prominent role in establishing an epigenetic cellular memory for cold-regulated expression states of *AGL19* and *FLC*. Finally, the similar network topology of the apparently independently evolved vernalization pathways of grasses and *Arabidopsis* is discussed.

Key words: *AGL19*, *Arabidopsis*, chromatin, epigenetics, *FLC*, flowering time, polycomb, PRC2, vernalization.

Introduction

Early observations reported that prolonged exposure to low temperatures can accelerate flowering in a broad range of plant species (for a review, see Chouard, 1960). This effect is termed vernalization, and constitutes a major determinant in the switch from vegetative to reproductive development. For non-perennials in temperate climates,

where the winter season lasts for several months, it is crucial that flowering occurs at the appropriate time, such as in early spring when environmental conditions favour reproductive success. In order to cope with this challenge, plants have devised vernalization mechanisms whereby cold is used as an enabling signal to induce the competence to flower.

One of the distinguishing features of vernalization is the uncoupling between stimulus and effect (Chouard, 1960). This uncoupling is both temporal, because often several months separate the initiation of the vernalization response and the actual transition to flowering, as well as developmental, because even imbibed seeds can become vernalized and retain the vernalized state throughout development until the adult phase. Although many of the physiological aspects of vernalization are still elusive, much progress has been achieved recently at deciphering the molecular basis of the underlying cellular-memory mechanism(s).

In *Arabidopsis thaliana*, the vernalization requirement is largely conferred by the MADS-box gene *FLOWERING LOCUS C (FLC)* (Michaels and Amasino, 1999; Sheldon *et al.*, 1999), which is in most vernalization-requiring accessions transcriptionally activated by *FRIGIDA (FRI)* (Napp-Zinn, 1957; Michaels and Amasino, 1999; Sheldon *et al.*, 1999; Johanson *et al.*, 2000). *FLC* then acts both in leaves and in the apical meristem to repress downstream floral integrators such as *FT* and *SOC1*, thereby acting as a floral repressor to delay flowering (Helliwell *et al.*, 2006; Searle *et al.*, 2006).

FLC is not exclusively regulated by vernalization (Fig. 1). A substantial number of additional unrelated positive and negative regulators have been described (for reviews, see Simpson, 2004; He *et al.*, 2005; Quesada *et al.*, 2005; Sung and Amasino, 2006; Schmitz and Amasino, 2007).

Vernalization acts at the epigenetic level to stably reduce *FLC* expression (for a review, see Schmitz and

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Abbreviations: CARG-box, CC(A/T)₆GG motif, MADS-domain protein-binding element; H3K27me₃, histone 3 lysine 27 trimethylation; LD, long days; PRC2, polycomb repressive complex 2; SD, short days.

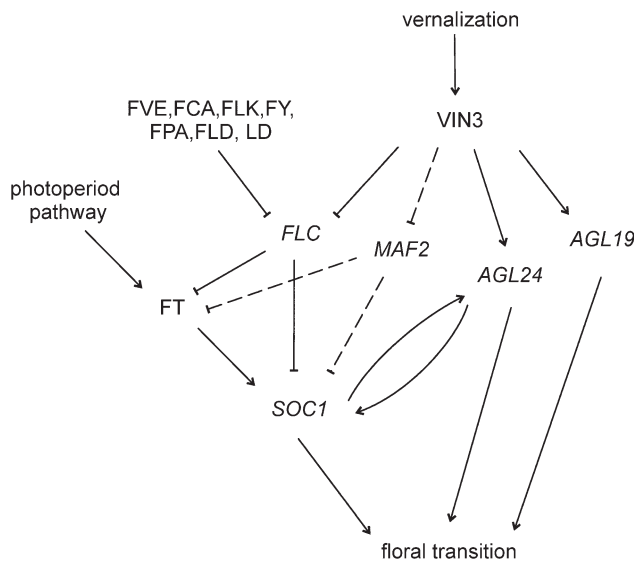


Fig. 1. The vernalization pathway in *Arabidopsis*. Three independent molecular vernalization responses have been identified in *Arabidopsis*, defining the *FLC*-, *AGL24*-, and *AGL19*-branches. Similar to *FLC*, its homologue *MAF2* might act to repress *SOC1* and *FT*, but this is not firmly established. Downstream factors of *AGL19* and *AGL24* remain unidentified. It is possible that, at least in part, *AGL24* acts through *SOC1*, because positive regulation between *AGL24* and *SOC1* occurs. Dashed lines represent hypothetical genetic links.

Amasino, 2007), and this effect overrides other types of regulation such as by the autonomous pathway. Once *FLC* is repressed by vernalization, it can only be reactivated in the next generation. The mechanism of *FLC* repression during vernalization has been studied extensively. The most upstream molecular effect of vernalization is induction of expression of the PHD-domain protein *VIN3* (Sung and Amasino, 2004). *VIN3* is only expressed at low temperatures, and both transcript and protein levels increase gradually with the length of exposure. Once sufficient amounts of *VIN3* protein are present, the cold signal is transduced into chromatin modifications at the *FLC* locus.

It has been reported that *VERNALIZATION 2* (*VRN2*), a homologue of one subunit of metazoan Polycomb-group Repressive Complex 2 (*PRC2*), is required for epigenetic maintenance of *FLC* silencing after vernalization (Gendall *et al.*, 2001; Bastow *et al.*, 2004). More recently, a *PRC2*-like complex including at least the *VRN2*, *FERTILIZATION INDEPENDENT ENDOSPERM* (*FIE*), and *CURLY LEAF* (*CLF*)/*SWINGER* (*SWN*) subunits has been reported to associate with *VIN3* at the *FLC* locus (Wood *et al.*, 2006). *PRC2* complexes are conserved between plants and animals, and usually have a histone methyltransferase activity that deposits histone 3 lysine 27 trimethylation (*H3K27me3*) marks on target genes (for a review, see Schwartz and Pirrotta, 2007). Increased levels of *H3K27me3* can be observed at *FLC* following vernalization (Shindo *et al.*, 2006; Sung *et al.*, 2006b;

Finnegan and Dennis, 2007; Greb *et al.*, 2007). Subsequently, *H3K27me3* is thought to recruit additional repressors such as *VRN1* and *LHP1*, which together assist in the maintenance of a stably silenced state (Levy *et al.*, 2002; Mylne *et al.*, 2006; Sung *et al.*, 2006a). Notably, it has long been known that vernalization requires mitotic activity (Wellensiek, 1962, 1964), and a recent report suggests that both *H3K27me3* at *FLC* and *FLC* repression cannot be efficiently maintained in non-dividing tissue after transfer to ambient temperature (Finnegan and Dennis, 2007).

Much of the knowledge on the molecular basis of vernalization has come from the studies of *FLC* regulation, and it is established that *FLC* is responsible for a considerable part of the vernalization response in *Arabidopsis*. Nonetheless, an *FLC*-independent vernalization response exists, because *flc* null mutants still have a vernalization-sensitive phenotype (Michaels and Amasino, 2001; Moon *et al.*, 2005). In the *flc* null mutant, both *FT* and *SOC1* are up-regulated after vernalization and this suggests that *FLC*-dependent and -independent vernalization branches share common targets (Moon *et al.*, 2005) (Fig. 1). Here, the current knowledge about the *FLC*-independent vernalization response is reviewed.

FLC*-independent responses in *Arabidopsis

***FLC*-related repressors of flowering**

Arabidopsis *FLC* is a member of a small family of closely related MADS-domain proteins. Besides *FLC*, this family contains the five MADS AFFECTING FLOWERING (*MAF*) proteins with 53–87% identity (Bodt *et al.*, 2003; Ratcliffe *et al.*, 2003). *FLOWERING LOCUS M* (*FLM*), also called *MAF1* is a repressor of flowering (Ratcliffe *et al.*, 2001; Scortecci *et al.*, 2001), and repression of *FLM* seems to contribute to the acceleration of flowering by elevated growth temperatures (Werner *et al.*, 2005; Li *et al.*, 2006). It is possible that all *MAF* proteins function as repressors of flowering (Ratcliffe *et al.*, 2003), but this hypothesis needs experimental testing. So far, a role for *MAF1* in the vernalization response has not been reported, but the recent findings that *MAF1* can be suppressed by vernalization (Sung *et al.*, 2006b) might bring new insights into *MAF1* functions.

FLC and some of the *MAF* genes share several regulators, such as *VIP5* and *ELF8/VIP6* (He *et al.*, 2004; Oh *et al.*, 2004) or *ESD1* (Martin-Trillo *et al.*, 2006). In addition, plants with reduced DNA methylation have reduced expression of *FLC* and *MAF1-5*, suggesting that DNA methylation alters the expression of a common *trans*-acting regulator (Finnegan *et al.*, 2005). By contrast, some other regulators were found to affect only a subset of the *FLC/MAF* genes (He *et al.*, 2004; March-Diaz *et al.*, 2007), but it is possible that, at least in some cases,

this reflects assay resolution rather than true biological differences. Similar to *FLC*, *MAF* genes are usually regulated by vernalization. Vernalization represses *MAF1*, *MAF2*, and *MAF3*, but it induces *MAF5* and does not strongly affect *MAF4* (Ratcliffe *et al.*, 2003; Sung *et al.*, 2006b). Furthermore, some natural variability occurs in the regulation of *MAF* expression, because differences between Col and two late-flowering accessions (Pitztal and Stockholm) have been reported (Ratcliffe *et al.*, 2003). Although H3K27me3 has been found to be essential for *FLC* repression by vernalization, it is not clear whether this histone mark is involved in the regulation of *MAF* genes as well. Examination of published data about genome-wide H3K27me3 distribution (Zhang *et al.*, 2007) revealed that *MAF4* and *MAF5* but not *MAF1*, *MAF2*, or *MAF3* are decorated with H3K27me3 (Fig. 2). However, in contrast to *FLC*, which is covered over its entire length by H3K27me3, *MAF4* and *MAF5* carry H3K27me3 only in certain regions. It should be noted that this genome-wide dataset is based on non-vernalized seedlings and that *FLC* carries considerable H3K27me3 even under these conditions. Because it is possible that after vernalization H3K27me3 marks accumulate also at *MAF1*–*MAF3*, more directed experiments are needed to address the potential involvement of this chromatin mark in *MAF* regulation.

In addition to *FLC* and *MAF1/FLM*, mutant studies have so far only revealed a function for *MAF2*. *maf2* mutants flower slightly earlier than wild type, but still retain a normal response to 6 weeks of vernalization (Ratcliffe *et al.*, 2003). However, if plants were submitted to only 10 d of cold, which is insufficient to elicit

a vernalization response in the wild type, a significant acceleration of flowering was observed. In fact, in *maf2* mutants a 21 d cold treatment can accelerate flowering to a similar extent as an 85 d cold treatment in wild type, suggesting that *MAF2* might regulate the delayed establishment of vernalization (Fig. 3). Such a specific function could prevent, for example, a few days of cold in autumn triggering precocious flowering during winter. This induction of flowering by short periods of low temperature seems to be independent of *FLC* as no significant decrease in *FLC* expression could be detected after 10 d of cold treatment (Ratcliffe *et al.*, 2003). Furthermore, *35S::MAF2* plants are unable to respond to vernalization due to continuous *SOC1* repression, even when *FLC* expression is normally reduced (Ratcliffe *et al.*, 2003). It is possible that an ancestor of the *FLC/MAF* family was a repressor of *SOC1* and that at least *MAF2* and *FLC* retained this original function. It will be important to establish if *MAF3*–*5* also repress *SOC1* expression and flowering.

The flowering promoter *AGL24*

Arabidopsis AGAMOUS-LIKE 24 (AGL24) and its paralogue *SVP* belong to the ancient *StMADS11* clade of MADS-box genes (Bodt *et al.*, 2003). Curiously, the separation of the *AGL24/SVP* branch involved strong positive Darwinian selection, and the same was observed for *FLC*-like genes (Martinez-Castilla and Alvarez-Buylla, 2003). Thus it seems that flowering time control is a specialized function acquired separately in different

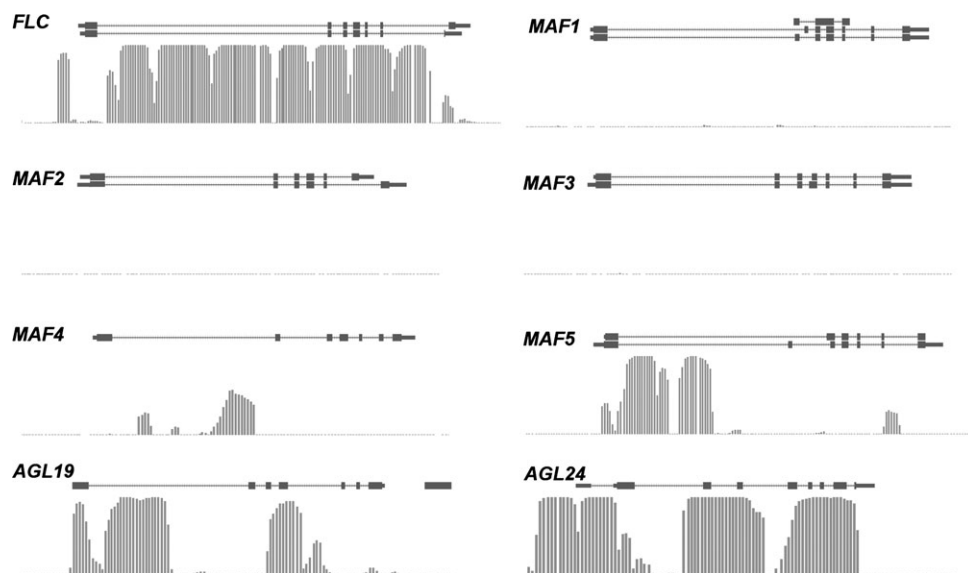


Fig. 2. Distribution of H3K27me3 marks on vernalization-related loci in *Arabidopsis*. Whole-genome analysis of H3K27me3 on wild-type seedlings (Zhang *et al.*, 2007) shows that *AGL19*, *AGL24*, *MAF4*, *MAF5*, and *FLC* all carry this repressive mark, although with very distinct profiles. Whereas for *AGL19*, *MAF4*, and *MAF5* the methylation is restricted to one or two defined regions, in both *FLC* and *AGL24*, H3K27me3 covers nearly the entire locus. Experimental evidence for a function of H3K27me3 exists only for *FLC* and *AGL19*. The lines represent gene models and the bars indicate the probability of the deposition of H3K27me3 at a particular position.

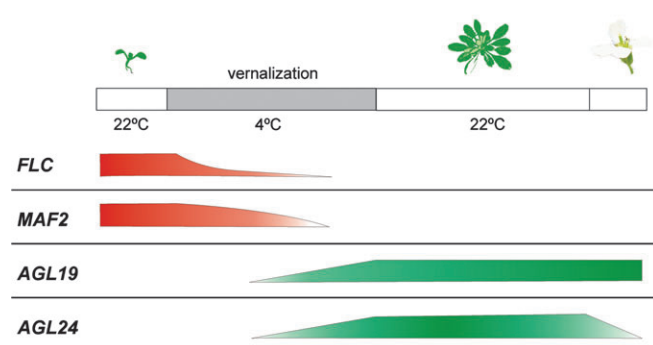


Fig. 3. Vernalization-regulated genes respond to different lengths of exposure to cold. The flowering activators *AGL24* and *AGL19* require long cold exposures to be activated, and are mostly functional from the late stages of vernalization on. The flowering repressors *FLC* and *MAF2* function before and during early stages of vernalization. *MAF2* has been proposed to prevent induction of flowering by short periods of cold, which could repress *FLC* (Ratcliffe *et al.*, 2003). The upper bar represents the different events in the plant development until flowering; red represents repressive functions and green activating functions; the shape is intended to depict gene function in relation to the duration of the cold period.

MADS-box gene lineages, and maintained most likely through its direct impact on plant fitness (Martinez-Castilla and Alvarez-Buylla, 2003).

AGL24 functions as an activator of flowering in response to vernalization (Yu *et al.*, 2002; Michaels *et al.*, 2003). *agl24* mutants are late-flowering, and this phenotype is only slightly suppressed by vernalization much as in *soc1* mutants. *SOC1* and *AGL24* activate each other's expression but can promote flowering independently as well (Yu *et al.*, 2002; Michaels *et al.*, 2003). Furthermore, unlike *SOC1*, *AGL24* is activated following vernalization in an *FLC*-independent manner.

In addition to its role in flowering-time control, *AGL24* has other developmental functions. Over-expression of *AGL24* is correlated with several floral abnormalities consistent with a role in establishing inflorescence meristem identity (Yu *et al.*, 2004). In fact, *AGL24* is generally expressed in vegetative organs before the floral transition, but gets progressively cleared as floral development proceeds and eventually becomes confined to the two inner whorls of the flower, the carpels and stamens (Michaels *et al.*, 2003; Yu *et al.*, 2004; Gregis *et al.*, 2006). Recently, it has been shown that repression of the flowering time genes *AGL24*, *SVP*, and *SOC1* by AP1 is an essential step in the establishment of floral meristem identity (Liu *et al.*, 2007). The emerging picture is that the same developmental regulators can assume distinct roles at different moments in the plant's life cycle.

The flowering promoter *AGL19*

Recently, another *FLC*-independent branch of the vernalization pathway in *Arabidopsis* was identified (Schönrock *et al.*, 2006). Again, the key regulator responding to the

vernalization treatment is a MADS-box gene, a close homologue of *SOC1* from the *TM3*-clade—*AGAMOUS-LIKE 19* (*AGL19*).

Initially described as root-specific, a novel role was assigned to *AGL19* after it had been found to be involved in controlling the flowering transition. When ectopically expressed, *AGL19* is a potent activator of flowering, but unlike *AGL24* only mild floral abnormalities were observed. This suggests that *AGL19* has a more restricted role in flowering time control. Also, in contrast to *AGL24*, *AGL19* does not affect *SOC1* levels, indicating that it probably acts independently of *SOC1* (Schönrock *et al.*, 2006). *AGL19* and *SOC1* share a conserved similar CArG-box (for CC-A rich GG) motif in their upstream regions. *FLC* binds the *SOC1* CArG-box and represses transcription (Hepworth *et al.*, 2002). In *AGL19*, the CArG-box sequence differs in a nucleotide that is essential for the *FLC*-binding to the *SOC1* promoter (Schönrock, 2006). Thus, it is likely that *FLC* cannot bind and repress *AGL19*. Indeed, *AGL19* expression levels are independent of *FLC*. Furthermore, genetic evidence also supports an *FLC*-independent function of *AGL19* because the double mutant *agl19 flc* has an additive impairment of the vernalization response (Schönrock *et al.*, 2006).

Interestingly, although *AGL19* acts as an activator of flowering, it shares a common regulatory mechanism with the flowering repressor *FLC*. Both are regulated at the chromatin level by vernalization in a *VIN3*-dependent manner. Chromatin immunoprecipitation assays demonstrated that *AGL19* chromatin is enriched in repressive H3K27me3 before, but much less after, vernalization (Schönrock *et al.*, 2006). While *FLC* is permanently silenced after vernalization, *AGL19* is temporarily silenced before vernalization. It has been reported that *FLC* silencing involves not only H3K27me3 but also repressive H3K9me2 marks (Bastow *et al.*, 2004; Sung and Amasino, 2004). By contrast, H3K9me2 marks could not be found on *AGL19* chromatin. In plants, H3K9me2 is usually associated with stable heterochromatic silencing while H3K27me3 is usually associated with more transient euchromatic silencing (Fuchs *et al.*, 2006), and it is possible that the stable *FLC* silencing involves additional, heterochromatin-like mechanisms not needed for the transient *AGL19* silencing.

H3K27me3 is thought to be deposited by PRC2-like complexes, and H3K27me3 at *AGL19* is most likely deposited by a complex of EMF2, CLF/SWN, FIE, and MSI1 (Schönrock *et al.*, 2006). By contrast, H3K27me3 at *FLC* is most likely deposited by a complex of VRN2, CLF/SWN, FIE, and possibly *VIN3* (Wood *et al.*, 2006) (Fig. 4). How the same signal, i.e. prolonged cold, can differentially affect distinct PRC2-like complexes is not known. It is possible that *VIN3* assists in assembling the VRN2 complex on *FLC* (Wood *et al.*, 2006). Because *VIN3* is needed for *AGL19* regulation as well, it would be

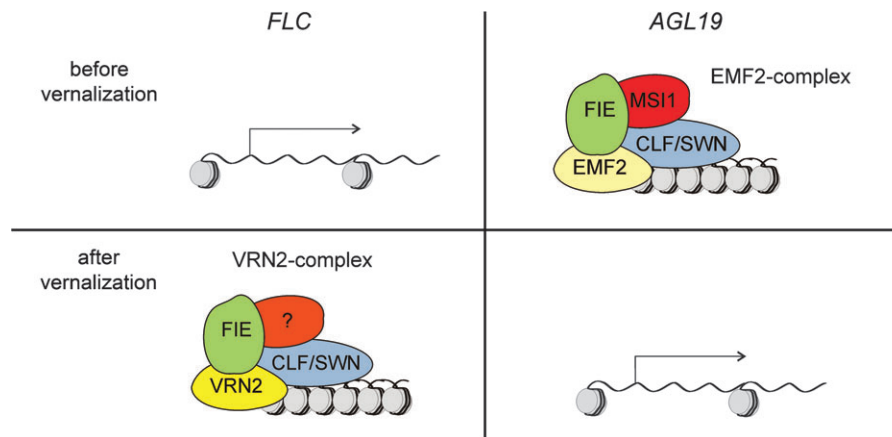


Fig. 4. Distinct polycomb-group complexes are required for vernalization-dependent regulation of *AGL19* and *FLC*. *AGL19* and *FLC* have opposite roles in promoting the switch to flower development and are differentially affected by vernalization, but both are regulated by polycomb-group complexes. For *FLC*, vernalization is thought to initiate recruitment of the VRN2 complex to *FLC* chromatin, leading to stable repression. For *AGL19*, vernalization is thought to initiate dissociation of the EMF2 complex from *AGL19* chromatin enabling gene expression.

highly interesting to investigate whether VIN3 could mediate disassembly of the EMF2 complex associated with *AGL19*.

Clearly, many details of the cellular memory of vernalization in *Arabidopsis* still need to be discovered, but it is exciting to see that stable gene repression by PRC2-complexes is a recurrent scheme. Notably, similar to *FLC* and *AGL19*, *AGL24* is also decorated with H3K27me3 marks, which are most likely deposited by a PRC2-like complex (Fig. 2). The prominent role of PRC2-like complexes and the H3K27me3 modifications raise the question whether other species use similar mechanisms in their vernalization response.

Vernalization pathways in other species

Initially, *FLC*-like genes were believed to be restricted to the Brassicaceae (Becker and Theissen, 2003), but recent work by Reeves *et al.* (2007) suggests that the strong positive Darwinian selection acting on these genes might have compromised their identification in other species and that the *FLC* clade actually originated early in the diversification of the eudicots. Reeves *et al.* (2007) identified the sugar beet (*Beta vulgaris* ssp. *vulgaris*) *FLC* homologue *BvFLC*. *BvFLC* is repressed by extended cold and delays flowering when expressed in transgenic *Arabidopsis* plants. Further research is needed to clarify how important *FLC*-like genes are for vernalization responses in non-Brassicaceae species.

The process of vernalization was first discovered in grasses, where vernalization is of great agronomic importance (for historical reviews, see Chouard, 1960; Amasino, 2004). Interestingly, many grasses are short-day–long-day (SD-LD) plants that need to be exposed first to short-day photoperiods and subsequently to long-day photoperiods to flower efficiently (Heide, 1994). In many

winter varieties, the initial SD treatment can substitute for the effect of prolonged cold on the induction of the competence to flower (McKinney *et al.*, 1935; Evans, 1987).

Much has been learned about the genetics and molecular mechanisms of vernalization responses in grasses (for a review, see Trevaskis *et al.*, 2007a). Work in barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) led to a model of vernalization that includes four central genes: *VRN1*, *VRN2*, *VRN3*, and *VRT2*, and genetic data strongly substantiate the importance of grass *VRN1-3* for vernalization (Fig. 4). Importantly, despite identical names, wheat and barley *VRN1* (also called *TmAPI1/TaVRT-1* and *HvVRN1*, respectively) and *VRN2* (also called *TmZCCT1* and *HvZCCTa/HvZCCTb*, respectively) do not share any sequence similarity with *Arabidopsis VRN1* and *VRN2*. Instead, grass *VRN1* is a homologue of the meristem identity MADS-domain protein APETALA1 (AP1) in *Arabidopsis* (Schmitz *et al.*, 2000; Danyluk *et al.*, 2003; Trevaskis *et al.*, 2003; Yan *et al.*, 2003; von Zitzewitz *et al.*, 2005), and grass *VRN2* shares the CO, CO-like, and TOC1 (CCT) domain with the flowering time regulator CONSTANS (CO) from the photoperiod pathway of *Arabidopsis* (Yan *et al.*, 2004). Grass *VRN3* is a homologue of *Arabidopsis* FT, a major component of florigen in *Arabidopsis* (Yan *et al.*, 2006). In grasses, *VRN1* and its homologue FUL are thought to be direct activators of flowering (Preston and Kellogg, 2007), and diploid einkorn wheat *T. monococcum* mutants that lack *VRN1* (*TmAPI1*) do not flower (Shitsukawa *et al.*, 2007). The vernalization response in wheat might include yet another gene, *VRT2*, a homologue of the *Arabidopsis* flowering-time genes *AGL24* and *SVP*. *VRT2* binds to the *VRN1* promoter *in vitro*, and can recruit *VRN2* (Kane *et al.*, 2005); *VRT2* and *VRN2* together can repress *VRN1* (*TaVRN1*) in a tobacco reporter assay (Kane *et al.*, 2007).

Both vernalization and SD photoperiods can repress *VRN2* and thus lift *VRN1* repression (Yan *et al.*, 2004; Dubcovsky *et al.*, 2006). However, for efficient activation of *VRN1*, *VRN3* is also needed. *VRN3* is repressed by *VRN2* and activated by LD photoperiods (Yan *et al.*, 2006). According to this model, vernalization or SD photoperiods are needed to repress *VRN2* and thus lift the repression from *VRN3* and *VRN1*. If subsequently LD photoperiods are present, *VRN3* will activate *VRN1* to induce flowering.

In this model, the signalling network of vernalization in grasses has the same topology as the FLC network in *Arabidopsis*: Two activators (FT/SOC1 and *VRN3*/*VRN1*), which transduce the LD signal for induction of flowering, are repressed by a negative regulator (FLC and *VRN2*) (Fig. 5). The two activators belong to the same protein families in *Arabidopsis* and grasses, respectively: FT and *VRN3* are both Raf kinase inhibitor-domain proteins (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999; Yan *et al.*, 2006), and SOC1 and *VRN1* are both MADS-domain proteins (Borner *et al.*, 2000; Lee *et al.*, 2000; Danyluk *et al.*, 2003; Trevaskis *et al.*, 2003; Yan *et al.*, 2003). By contrast, the repressor that is under negative control by vernalization differs: FLC is a MADS-domain protein (Michaels and Amasino, 1999; Sheldon *et al.*, 1999), but *VRN2* is a CCT-domain protein (Yan *et al.*, 2004). This reflects the likely evolutionary history of the signalling networks: FT-like proteins seem to be ancient floral activators that transmit day-length signals in *Arabidopsis* (LD plant), rice (SD plant), and poplar (perennial tree) but

function also in tomato (day-neutral plant) (Bohlenius *et al.*, 2006; Lifschitz and Eshed, 2006; Corbesier *et al.*, 2007; Jaeger and Wigge, 2007; Mathieu *et al.*, 2007; Tamaki *et al.*, 2007). It is believed that vernalization requirement developed independently in *Arabidopsis* and grasses. In the former, a MADS-box transcription factor evolved to repress FT (and *SOC1*) in the absence of vernalization. In the latter, a CCT-domain protein evolved to repress *VRN3* (and *VRN1*) in the absence of vernalization.

An alternative model suggests that vernalization in grasses acts primarily on *VRN1*, which represses *VRN2* (Fig. 5) (Trevaskis *et al.*, 2006, 2007a, b). Given that FT is a mobile signal and that FLC acts both in the leaves and in the meristem, it will be important to establish in grasses in which organs and tissue *VRN1*, *VRN2*, and *VRN3* function. In addition, the role of *VRT2* and its homologues needs further investigation. Recently it was proposed that barley *VRT2*-like genes do not participate in vernalization-mediated repression of *VRN1* but rather function in a similar way to *Arabidopsis* *AGL24* and *SVP* to inhibit floral meristem identity (Trevaskis *et al.*, 2007b).

Another open question is the nature of the cellular memory of vernalization in grasses. While chromatin-based mechanisms, which involve PRC2-like complexes, are needed for the maintenance of silencing in the *Arabidopsis* *FLC* and *AGL19* branches, it is not clear to which extent such mechanisms control vernalization in grasses. Two lines of evidence suggest that this might be the case: First, three wheat *VIL3*-like (*VIL*) genes (*TmVIL1–3*) were described (Fu *et al.*, 2006). Similar to *Arabidopsis* *VIN3*, *TmVIL1–3* transcripts accumulate after 4–6 weeks of cold treatment, but return rapidly to pre-vernalization levels after the shift to ambient temperatures. It remains to be tested whether *VIL* proteins mediate the vernalization response in grasses. Second, transcriptional profiling of the vernalization response in perennial rye grass (*Lolium perenne*) identified not only a MADS-box gene and a *VRN2*-like CCT-domain gene but also a JUMONJI (*JmjC*)-like gene, *LpJMJC* (Ciannamea *et al.*, 2006). Although *JmjC*-domain proteins have not yet been found in the *Arabidopsis* vernalization response, the *JmjC*-protein REF6 is a repressor of *FLC*, possibly by modifying *FLC* chromatin (Noh *et al.*, 2004). *LpJMJC* is not a close homologue of REF6, but it may also act through chromatin remodelling. This idea is supported by the recent finding that *JmjC*-domain proteins are often histone-demethylases (Klose *et al.*, 2006). More research is needed to establish whether *JmjC* proteins such as *LpJMJC* mediate epigenetic regulation of vernalization-responsive genes in grasses.

Conclusions

The ability to be vernalized is an important adaptive trait in plants. Evolution found multiple answers to the old

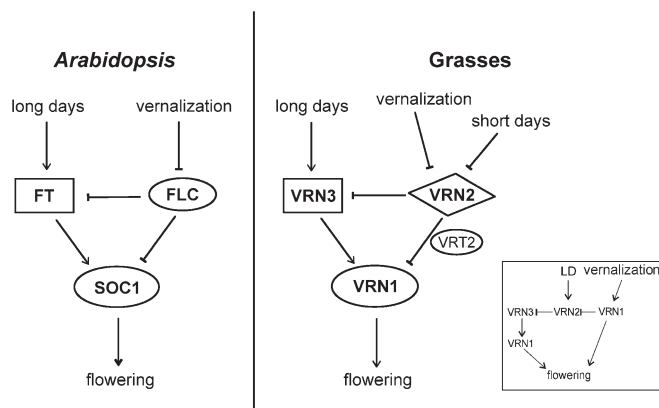


Fig. 5. Common topologies for vernalization pathways in *Arabidopsis* and in grasses. Vernalization networks evolved independently in distant plant groups, but have similar topology in *Arabidopsis* and the grasses. In both cases, two activators are sensitive to long-day photoperiod signals (FT/SOC1 and *VRN3*/*VRN1*), and are repressed by a negative regulator (FLC and *VRN2*). In *Arabidopsis* and grasses, the activator functions are executed by one FT-like and one MADS-domain protein. The repressor is the MADS-domain protein FLC in *Arabidopsis* and the CCT-domain protein *VRN2* in grasses. The shapes represent protein families: boxes for FT-like Raf-kinase-inhibitor-domain proteins, ovals for MADS-domain proteins, and diamonds for CCT-domain proteins. Inset: an alternative model to explain the vernalization response in grasses was proposed, where the primary target of cold is *VRN1* and not *VRN2* (Trevaskis *et al.*, 2007).

question: ‘how to check that the winter is over and that a “better life” can begin?’ (Becker *et al.*, 2003). Mechanisms of *FLC*-dependent vernalization are understood best, but it remains to be tested how widely they are used outside of the Brassicaceae. At least in grasses a different vernalization pathway evolved. Interestingly, even within a single species, vernalization signalling can follow multiple branches. It is possible that, after the first vernalization pathways evolved, selection favoured the addition of more robust branches to the vernalization signalling network. In the case of *Arabidopsis*, vernalization involves *FLC*-, *AGL24*-, and *AGL19*-dependent branches, and it is currently not clear which of the three is most ancient and which is most recent. Despite the multiple appearances of pathways for vernalization during evolution, there are recurrent themes. First, vernalization networks consist of similar network motifs and are of similar topology. This suggests that certain network structures evolve easily and give a robust performance. Second, epigenetic memory mechanisms are used both in the *FLC*- and the *AGL19*-branches of the *Arabidopsis* vernalization pathway. Such epigenetic mechanisms ideally serve the purpose of stable maintenance of previously established gene expression states. It will be exciting to see whether epigenetic mechanisms such as PRC2-mediated gene silencing participate in other vernalization pathways as well.

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